REMARKS

Claims 1-3, 8-10, 18-20 and 38-49 are pending.

Applicants thank the Examiner for withdrawal of the rejection of *independent* claim 18, under 35 U.S.C. § 102(e), as being anticipated by Hudziak (U.S. Patent No. 6,399,063).

Applicants acknowledge the Examiner's new ground of rejection relating to Sequence Rules with respect to Figures 1 and 8, as well as the inadvertent error in SEQ ID NO:2, and have responsively amended the Specification and Sequence Listing accordingly to obviate these issues.

Applicants acknowledge the Examiner's maintained rejections of claims 1-3, 8-10, 18-20 and 38-49, under 35 U.S.C. 112 ¶1, based on alleged lack of written description and enablement. Applicants provide arguments and claims amendments to address these issues.

Applicants, to facilitate prosecution, have revised the Sequence Listing to explicitly recite the various species of applicants' originally filed SEQ ID NOS:1 and 2.

No new matter has been added.

FORMALITIES

Sequence Listing

The Examiner has indicated that the Specification does not comply with sequence requirements in view of (**i**) the lack of sequence identifiers in the Brief Description for Figures 1 and 8, and (**ii**) the inadvertent error at position 389 in SEQ ID NO:2 (Office Action of 16 July 2004, page 3).

With respect to (**i**), applicants have amended (*see* above-described Amendments to Specification) the Brief Description relating to Figures 1 and 8 to include respective sequence identifiers conforming to the presented nucleic acid (SEQ ID NOS:16, 17 and 18) and polypeptide (SEQ ID NOS:11 and 14) sequences, and have enclosed CRF and paper copy of the amended SEQUENCE LISTING (attached hereto).

Specifically, the Brief Description relating to Figure 1 has been amended to recite: "SEQ ID NO:16" for the 287 nucleotide DNA sequence shown in the upper panel A of Figure 1; "SEQ ID

NO:17 (276 bp) for the 275 nucleotide insert, plus the immediately 5' "G" nucleotide, sequence portion of SEQ ID NO:10; and "SEQ ID NO:11 for the novel in-frame 79 amino acid sequence referred to that is encoded within SEQ ID NO:10. Correspondingly, SEQ ID NOS: 16, and 17 have been added to the SEQUENCE LISTING (SEQ ID NO:11 being already present in the listing).

Specifically, the Brief Description relation to Figure 8 has been amended to recite "SEQ ID NO:18" for the nucleotide sequence, and "SEQ ID NO:14" for the deduced amino acid sequence of the most common sequence. Correspondingly, SEQ ID NO:18 has been added to the SEQUENCE LISTING (SEQ ID NO:14 being already present in the listing).

Support for these amendments is inherent to originally submitted sequences of Figures 1 and 8, and no new matter has been added.

With respect to **(ii)**, the inadvertent recitation of "Asp" instead of the intended "Ser" at position 389 in SEQ ID NO:2 has been corrected to conform to the corresponding "Ser" residue 49 of applicants' originally filed SEQ ID NO:1, which corresponds to the relevant portion of SEQ ID NO:2. Additional support for this correction is found in originally filed Figures 1 and 8, which also conform to the art-recognized "Ser" residue at this sequence position.

No new matter has been added.

Applicants contend that the Specification and SEQUENCE LISTING are now in compliance with the sequence rules with respect to these issues, and respectfully request withdrawal of this objection.

Rejections under 35 U.S.C. § 112 ¶1 *(written description / enablement)*

Written description:

The Examiner maintained the rejection of claims 1-3, 8-10, 18-20 and 38-41, under 35 U.S.C. § 112 ¶1, alleging that the specification lacks sufficient written description of variants commensurate in scope to the claimed subject matter (Office Action of 16 July 2004, page 3, paragraph 5).

Specifically, the Examiner asserts that "Table 1 discloses individual variants, including one that is to be specifically excluded, but does not disclose all the possible variants that could be

predicted from the formula of SEQ ID NO:1." The Examiner further asserts that most of the specification and working examples are directed to teaching the biological function and activity of the excluded sequence, concluding that "the claimed subgenus is broader than what is disclosed in Table 1."

Applicants respectfully traverse this rejection, based on the fact that there is adequate written description to exclude the prior art Herstatin sequence, as well as describe the claimed genus.

First, as previously argued in the record, applicants' Example 11 explicitly discusses the excluded sequence in the context of the prior art (referencing Doherty et al., PNAS 1999) and goes on to "[a]dditionally" describe a genus of new polymorphisms in the context of Table 1 (Specification at page 32, lines 6-10; and see prior Clinton affidavit of record on this issue). Therefore, as previously argued, there is ample adequate description to reasonably convey that applicants had possession of a polymorphic genus excluding the particular prior art Herstatin sequence. Furthermore, the Examiner's previous new matter rejection with respect to the exclusion issue was withdrawn in response to the applicants' prior arguments of record. Therefore, applicants interpret that the Examiner is now questioning whether, despite having excluded the prior art sequence, applicants' written description extends beyond the individual variants listed in Table 1 to encompass compound variants. Applicants contend that it most certainly does.

The originally filed claims *explicitly* recite "SEQ ID NOS:1 and 2," and thus explicitly describe and show that all new individual and possible combinatorial variants thereof were within the inventors' conception at the time of filing. The description of Table 1 includes the words "for example" in describing particular polymorphisms (page 32, line 14), and additional states (page 32, line 23) that "<u>some</u> identified variants are described in Table 1" (emphasis added). Significantly, this open-ended language reflects the fact that ten (10) polymorphisms were found in sequencing the DNA of only 15 individuals, with the prior art polymorphism (variant 11) representing a *compound* double variant of new polymorphisms variants 3 and 10. Therefore, applicants not only had basis for conception of a broad genus comprising individual and compound variants, but also had possession of such a genus at the time of filing, and as originally claimed.

Applicants, in the interest of advancing the application to allowance, have nonetheless amended the claims to recite particular variants, thereby rendering this rejection moot.

Specifically, *independent* claims 1, 18 and 38 to recite "SEQ ID NOS:14 and 19-28, and fragments thereof of..." instead of "SEQ ID NO:1, and a fragment of SEQ ID NO:1 of...." Conforming amendments have been made to the relevant dependent claims. Additionally, applicants have amended *independent* claims 8, 18 and 39 to recite "SEQ ID NOS:15 and 29-38, and fragments thereof of..." instead of "SEQ ID NO:2, and a fragment of SEQ ID NO:2 of...." Conforming amendments have been made to the relevant dependent claims.

SEQ ID NO:14 corresponds to the wild-type or "most common sequence" of the intron-incoded ECDIIIa region of Herstatin as shown in Figure 8, and SEQ ID NOS:19-28 correspond, relative to the most common sequence, to the variants 1-10 of the intron-incoded ECDIIIa region of Herstatin as depicted in the Table of Example 11. SEQ ID NO:15 corresponds to the "most common sequence" of full-length Herstatin, and SEQ ID NOS:29-38 correspond, relative to this most common sequence, to variants 1-10 of full-length Herstatin. The claims, therefore, have been clarified to encompass the most common sequence and the individual variants. The Herstatin claimed in applicants' prior issued U.S. Patent No. 6,414,130, and pending application serial number 09/234,208 corresponds to variant 11 of Table 1 of Example 11 of the instant specification, and appropriately is not encompassed by the instant claims.

Support for these amendments is found in applicants' originally filed SEQ ID NOS:1 and 2 and in Table 1 and the description thereof, and in Figure 8. Applicants have correspondingly further amended the Sequence Listing (attached) to recite these individual species of originally filed SEQ ID NOS:1 and 2. No new matter has been added.

Second, applicants contend that the Examiner's assertion that "most of the specification appears to be directed to teachings concerning the biological function and activity of the excluded species," goes to scope of enablement of the claims, and is not relevant to sufficiency of written description. Nonetheless, to initially address the Examiner's comments, the originally filed claims explicitly encompass the inventors' conception that the discovered polymorphic variants have utility in pharmaceutical compositions, for example, in treating cancer cells expressing HER-2.

Furthermore, the application as originally filed provides a detailed structural and functional characterization of Herstatin, a naturally occurring inhibitor of HER-2 dimerization and other tyrosine kinase receptors, some of which are overexpressed in certain carcinomas. The application describes that when Herstatin from several individuals is isolated and sequenced, allelic variants of this protein are identified. The Examiner provides no reason to doubt that allelic variants of the same protein, in a population of normal human beings, function differently. To applicants' knowledge, none of the human beings from whom Herstatin was sequenced showed anomalies such as, for example, a predisposition to cancer characterized by overexpression of HER-2. Applicants, at the time of filing, clearly "possessed" several naturally occurring allelic variants of Herstatin, and applicants appreciated, described and claimed these allelic variants as having a specified binding affinity (at least 10^8 M^{-1}) for the extracellular domain of HER-2. The Examiner has provided no reason to doubt that the HER-2 binding affinity and inhibition of dimerization demonstrated for one Herstatin polymorphic variant (variant #11 from Table 1) would not apply to other allelic variants of the same protein, particularly given that each of the variants was isolated from a normal individual. This issue is addressed in more detail under the rejection on grounds of inadequate scope of enablement.

Applicants, therefore, respectfully request withdrawal of the Examiner's U.S.C. § 112 ¶1 written description rejection.

Enablement:

The Examiner maintained the rejection of claims 1-3, 8-10, 18-20 and 38-41, under 35 U.S.C. § 112 ¶1, alleging that the specification lacks sufficient enablement of variants commensurate in scope to the claimed subject matter.

Specifically, the Examiner asserts that while the specification teaches "amino acid sequences of a number of variants," it "fails to describe the biological activity of any variant within the scope of the claims," and that the only binding and tumor cell inhibition data are with respect to applicants' previously claimed Herstatin corresponding to excluded variant 11. Additionally, the Examiner points to literature references that allegedly teach how single amino acid substitutions can

lead to dramatic changes in biological activity, and further asserts that applicants' own "specification asserts that the variations in the amino acid sequence of Herstatin could lead to altered biochemical and biological properties among the variants" (citing the specification at page 16, lines 13-15) (Office Action of 16 July 2004, pages 4-5). The Examiner states that although the claimed variants "may possibly" have similar biological activity to previously claimed variant 11, their biological function is "unpredictable" in light of the uncertain relationship between primary amino acid sequence and protein function, concluding that determination of biological function and attendant use of each of the variants would require *undue experimentation* by one of skill in the art.

Applicants respectfully traverse this rejection, based on having established *utility* for an exemplary member of a group of naturally occurring allelic variants from normal individuals, and on the *ample guidance* by the specification in the form of representative working examples relating to the exemplary member in a context where the Examiner has not provided any evidence that the disclosed variants are not biologically active.

Utility. With respect to utility, the threshold is quite low, and all that needs to be established is success in achieving a useful result; all intended functions or operability under all conditions need not be established (*In re Brana*; *In re Marzocchi*). In this instance, one of the variants known to occur naturally in human beings, variant 11, clearly binds to and inhibits dimerization and tyrosine phosphorylation activity of HER-2. Variant 11 is not even the most common naturally occurring variant in normal human beings; the variant whose intron 8-encoded sequence is set forth in Figure 8 (*i.e.*, SEQ ID NO:14) is. Applicants have established that a naturally occurring splice variant of HER-2, Herstatin, which shows several allelic variations in a population of normal human beings, functions as an inhibitor of HER-2 by binding to its extracellular domain and inhibiting dimerization and tyrosine phosphorylation (specification at pages 30-31, Examples 9 and 10). Herstatin is a naturally occurring inhibitor of HER-2, a receptor whose over-expression has been implicated in some cancers. Therefore, a useful result, namely, regulation of HER-2 over-expression, for naturally occurring Herstatin in normal individuals has been obtained. Operability of all possible naturally occurring allelic variants of the same protein, Herstatin, isolated from normal individuals need not be established.

Alleged lack of enablement. The test for sufficient enablement is whether the amount of experimentation required by one of skill in the art to make and use the claimed subject matter is not *undue* (although a reasonable amount of experimentation is permitted). The test for undue experimentation requires an application of the factors set forth in *In re Wands*, cited by the Examiner.

The claims are directed to particular isolated (polypeptide) allelic variants of Herstatin and pharmaceutical compositions containing the isolated polypeptides. The specification teaches how to isolate and sequence each of the claimed variants. In addition, the specification teaches in great detail, and provides numerous working examples of how to isolate Herstatin, its structural characterization, the sequences of Herstatin that are involved in binding to the extracellular domain of the HER-2 receptor, and how to measure the binding affinity of Herstatin to the ECD of HER-2, inhibition of dimerization and inhibition of tyrosine phosphorylation activity of HER-2. These teachings and working examples, in light of the level of those of skill in the art, the knowledge of those of skill in the art regarding the structure and function of normal allelic variants of the same protein, the predictability of teachings regarding one normal allelic variant being applicable to other normal variants, leads to the conclusion that it would not require undue experimentation to make and use subject matter that is commensurate in scope with the claims.

Wands factors. Applicants address the Wands factors cited by the Examiner, and cite relevant sections of the specification:

Scope of the Claims: The claims are directed to isolated polypeptide allelic variants of Herstatin that bind to the ECD of HER-2 with specified binding affinity, and related pharmaceutical compositions. The claims are clearly within the scope of what is taught in the specification, *e.g.*, a genus of polymorphic variants that includes the particular claimed variants. The specification teaches that Herstatin, which is an autoinihibitor of HER-2, has numerous allelic variants in normal human beings (specification at pages 32-33, Example 11). The specification further teaches that each of these variants is isolated in the same manner (pages 25-27, Example 4), and provides detailed assays for their functional characterization (pages 30-31, Examples 8, 9 and 10). By following the teachings of the specification, one of skill in the art can readily make the claimed

variants and measure their binding activity. Therefore, the scope of the claims is commensurate with the teachings of the specification.

Teachings of the specification: The specification provides a detailed structural and functional characterization of a naturally occurring inhibitor of HER-2, Herstatin (Examples 1-10). The specification teaches how to isolate and identify various normally occurring allelic variants of Herstatin (pages 32-33, Example 11), and how to measure their biochemical and biological activity (pages 30-31, Examples 8, 9 and 10). The specification teaches the exact steps of how to isolate (Examples 1-4) and identify variants (Example 11) of Herstatin that bind to HER-2 with a specified binding affinity (at least 10^8 M^{-1}, as set forth in the claims).

The Examiner points to page 16, lines 13-15 of the specification, alleging that Applicants acknowledge how variations in the sequence of Herstatin could lead to altered biochemical and biological properties. The cited passage and the sentences following the same however merely recites that differences among the variants, such as size, electronegativity, or antigenicity, may impact the extent of cancer susceptibility, tumor progression or treatment protocols. There is no teaching in the specification that the claimed naturally occurring allelic variants of the same protein, Herstatin, in normal individuals would function in a qualitatively different manner, *i.e.,* enhance or have no effect on HER-2 activity rather than inhibit HER-2 activity. As taught by the specification, allelic variants of Herstatin all have the same function, namely, they bind to and inhibit HER-2 and related families of receptors. Moreover, as discussed above, the claims specify that the variants bind to HER-2 with a binding affinity of at least 10^8 M^{-1}, and the specification teaches how to assay for specific binding of a variant to HER-2.

Level of Skill in the Art: This is recognized to be high, as evidenced by the numerous publications of record in the file history and also by the courts.

Knowledge of those of Skill in the Art: At the time of filing of the application, a considerable amount was known about HER-2 and other tyrosine kinases, how several carcinomas are characterized by over-expression of the tyrosine kinases such as HER-2, the identification and isolation of Herstatin, its structural characterization and sequence analysis, how it is a splice variant of HER-2 containing intron-encoded sequence, how it functions as a naturally occurring

autoinhibitor of HER-2, and how to measure its binding and inhibitory activities (see, *e.g.*, Doherty et al., *PNAS USA* 96:10869-10874, 1999, and references discussed therein; and see the Background section of the present applicant at pages 1-2, citing and discussing references relating HER-2 overexpression with cancer, and discussing other HER-2 family members).

Working Examples: The working examples show how to isolate and sequence Herstatin, how to isolate various naturally occurring allelic variants in normal individuals, and how to measure HER-2 binding and inhibitory activities of Herstatin.

Specifically, Example 1 describes construction of cDNA libraries from SKOV-3 carcinoma cells, PCR amplification of cloned Herstatin cDNA, sequencing of the amplified Herstatin cDNA, and characterization of the structure of the Herstain cDNA and corresponding mRNA.

Example 2 describes characterization of exon sequences that flank the Herstatin ECDIIIa (intron 8) region, establishing Herstatin-encoding mRNA/cDNA structure.

Example 3 shows that the Herstatin ECDIIIa region (intron 8) is the only retained intron observed in the Herstatin mRNA.

Example 4 describes how to express and isolate and characterize Herstatin polypeptides.

Example 5 describes how to examine (*e.g.*, Northern blotting) and characterize cellular Herstatin mRNA species.

Example 6 describes how to analyze (*e.g.*, Northern analysis) and verify that Herstatin mRNA is expressed in *normal* human tissue.

Example 7 describes how to examine and characterize cellular expression of Herstatin polypeptides by Western analysis using Herstatin antisera.

Example 8 describes how to analyze and characterize the relative levels of Herstatin and HER-2 expression in various cells.

Example 9 describes how to analyze and measure specific binding of Herstatin and the Herstatin ECDIIIa region to HER-2 using anti-HER-2 and anti-ECDIIIa antisera in 'pull-down' assays and Western blots. This example also shows how to measure specific binding of Herstatin to HER-2 expressing cells, by Western blot analyses of cell extracts.

Example 10 describes how to measure and characterize the effects of Herstatin binding on HER-2 activation/tyrosine phosphorylation and related signal transduction. Additionally, the specification (at page 21, lines 10-28 and Figure 7) describes bioactivity in relation to SKOV-3 and 17-3-1 cancer cells, and shows how to test Herstatin for inhibition of cancer cell growth.

Example 11 describes the isolation and sequence characterization of Herstatin polymorphic/allelic variants, including a novel "most common sequence," from normal individuals. Table 1 lists some of the identified allelic variants in relation to the most common sequence that is shown in Figure 8. This example describes and teaches that naturally occurring polymorphisms from normal individuals include both single, and double/compound variants relative to the most common sequence, and that the previously claimed allelic variant 11 is actually a double variant.

Applicants emphasize that although the binding and inhibitory activities are demonstrated for one particular normal naturally occurring allelic variant (variant 11), it is applicable to the other identified variants of the same protein. Applicants are not required to provide data or illustrative examples of every embodiment that is within the scope of a claim (*In re Anderson*), and these are all naturally occurring allelic variants found in normal individuals. There is nothing of record to establish that these allelic variants do not have the same activity as the previously claimed variant 11 (Doherty et al., *PNAS USA* 96:10869-10874, 1999).

Notwithstanding the above, the Declaration under §1.132 discussed below demonstrates that by following the teachings of the specification, one of skill in the art can obtain variants of Herstatin that bind to and inhibit HER-2 in a manner that is comparable to other known variants.

Predictability: The Examiner cites several references in support of the proposition that a single amino acid substitution can alter biochemical and biological properties dramatically. That is however applicable in cases where a defect/disease is observed and can be attributed to the mutant. In this instance, no such anomaly was observed and all variants were identified in healthy, normal individuals. Given the knowledge of those of skill in the art and the teachings of the specification, it is predictable that naturally occurring allelic variants of the same protein in a normal human population would have the same function. None of the human beings from whom Herstatin was isolated showed an unusual predisposition to cancers characterized by over-expression of HER-2,

nor any other anomalies. Therefore, the teachings of the specification as exemplified with one variant of the same protein are applicable to the other variants. The disclosed allelic variants differ only in one or two amino acids from the prior art reference Herstatin variant 11, and it is fundamentally inappropriate to misconstrue the possibility of *altered* biochemical and biological properties among these polymorphic variants, as evidence that they have *no* binding or biological properties. The fact that variants may have *different* affinities, structure, electronegativity, antigenicity, etc. (specification at page 16, lines 19-35) is not relevant from the standpoint of a group of normal human allelic variants having a characteristic activity.

Conclusion: In conclusion, the claims specify that Herstatin allelic variants that bind to HER-2, and the specification teaches how to isolate, express and measure binding, HER-2 inhibition activity, and cancer cell growth inhibition activity of the allelic variants. The *scope of the claims*, which comprises a genus of allelic variants (same protein) is clearly within the scope of what is *taught in the specification*, which in addition to the *working examples* teaching isolation and a detailed structural and function characterization of Herstatin allelic variant 11, teaches isolation and characterization of a genus of alleles isolated from normal individuals. The fact that variant 11 is not presently claimed does not defeat the ample guidance and substantial enabling value of these working examples with respect to the other disclosed polymorphic variants. Moreover, the *level of skill in the art* is high, and a considerable amount of information relating to Herstatin and how it functions as a naturally occurring autoinhibitor of HER-2 was known in the art at the time of filing. Furthermore, the *predictability* factor is weighted in applicants' favor in view of the fact that the polypeptide variants are allelic variants isolated from normal individuals. Therefore, in light of the *Wands* factors as applied to the present claimed invention, applicants conclude that it would not require undue experimentation to make and use the claimed subject matter.

Section 1.132 Affidavit:

Notwithstanding the above, an Affidavit under 37 C.F.R. § 1.132 by Dr. Clinton is attached to evidence that the methods as claimed, operate as claimed. The Affidavit describes experiments that ~~closely~~ follow the teachings of the specification (and as described in Shamieh *et al.,* which is a

subsequent publication of the subject matter of this application). The Affidavit and Shamieh *et al.*, show that allelic variants of herstatin show specific binding to and inhibition of HER-2 and other ErbB family receptors.

Specifically, Figure 1 of the affidavit shows, using HER-2 overexpressing 3T3 cells, a direct comparison of HER-2 specific binding among: (**a**) the most common Herstatin form ("wt"; corresponding instant SEQ ID NOS:14 and 15); (**b**) the "L/N" variant (the previously disclosed and claimed variant 11 that contains two substitutions in the intron 8 encoded ECDIIIa domain; namely Pro to Leu (variant 3; SEQ ID NOS:21 and 31), and Asp to Asn (variant 10; SEQ ID NOS:28 and 38) at amino acids positions 6 and 73, respectively, relative to "wt"); and (**c**) an "R to C" variant that contains a substitution of Cys in place of Arg at amino acid position 17 of the ECDIIIa intron encoded region (the "R to C" variant is the subject of a more recent pending patent application). Expression and purification of these variants was performed as described in the specification, for example, at pages 25-27 and in Example 4. All three molecules show comparable strong, specific binding at nM concentrations.

Additionally, Figure 2 and Figure 3 of the affidavit show a direct bioactivity comparison of inhibition of the growth of DU145 prostate cancer cells that over-express the ErbB receptor family (including HER-2, EGFR and HER-3 receptors), among: "wt" (most common sequence); the "L/N","R to C", and "R to I" polymorphic forms of Herstatin. The results show that all three Herstatin allelic forms had strong, comparable activity in inhibiting the growth of this ErbB receptor family-expressing cancer cell line.

The results show that polymorphic forms of Herstatin, isolated and tested according to the teachings of the application, have strong, comparable activity in inhibiting HER-2 expressing cancer cells, as well as cancer cells expressing other ErbB family receptor members.

Finally, Shamieh *et al* show binding interactions of Herstatin and the intron-encoded domain thereof with receptors of the ErbB receptor family including HER-1 (EGFR), HER-2, HER-3, HER-4, and additionally to IGF-1R. Both "wt" Herstatin (SEQ ID NO:15) and the corresponding intron-encoded portion thereof (SEQ ID NO:14) bind at nM concentrations to HER-2 and EGFR (and also to ΔEGF-R, HER-4 and IGF-1R). Additionally, the full-length Herstatin, comprising the "R to I"

ECDIIIa sequence variant (Arg –> Ile at amino acid position 31 of the ECDIIIA region) has bioactivity against ErbB family receptor overexpressing DU145 prostate cancer cells that is comparable to that of the "wt" molecule.

Applicants, therefore, respectfully request withdrawal of the Examiner's 35 U.S.C. § 112 ¶1 enablement rejection of claims 1-3, 8-10, 18-20 and 38-49.

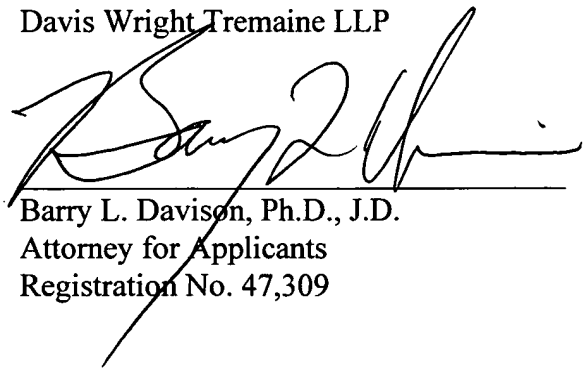
CONCLUSION

In view of the foregoing amendments and remarks, applicants respectfully request entry of the present Amendment and allowance of all claims as provided herein above.

The Examiner is encouraged to phone applicants' attorney, Barry L. Davison, to resolve any outstanding issues and expedite allowance of this application.

Respectfully submitted,

Davis Wright Tremaine LLP



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